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14. ABSTRACT
Research progress over the past year focused on imaging of lens fiber cell structure using confocal laser scanning microscopy (CLSM), and type IV collagen structure using cryo-electron microscopy and tomography (cryo-EM and ET). The CLSM promises to generate good structural identification of lens fiber cells, while the cryo-EM and ET have not yet positively identified type IV collagen structure of the lens capsule. The collaboration with Dr. Vernerey of CU Boulder on large deformation Lagrangian-Eulerian computational modeling of the lens will be extended to three-dimensions (3D) and ultra-structurally based modeling of capsule and fiber cells. Such modeling could be extended to the whole eye globe, given the ease of using Eulerian-based methods in large deformation computational mechanics and solid-fluid interaction. Tracking interfaces is the challenge, and such details are being investigated as part of the research.

15. SUBJECT TERMS
lens capsule ultrastructure, lens fiber cell ultrastructure, Eulerian-Lagrangian large deformation computational modeling of lens

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1 Introduction

In the life of a combat soldier, traumatic cataract in ocular lenses may result from blast loading, whereby (i) the lens capsule (Fig.1) is perforated by intraocular foreign bodies (IOFBs [Walter, 1962, Mader et al., 1993, Parver et al., 1993, Wong et al., 1997, Mader et al., 2006, Weichel and Colyer, 2008]) which in turn damage the lens fiber cells, (ii) the lens is loaded fluid dynamically by the surrounding aqueous and vitreous humors [Banitt et al., 2009] (see Fig.1), and/or (iii) the lens internal substance (crystallins lens fiber cells) is stressed by the passing shock wave. Traumatic cataract can result in a partially or fully clouded lens, complete dislocation of the lens (floating between aqueous and vitreous humors, see Fig.1), or zonule rupture such that partial or full vision loss may occur. The mechanisms of traumatic cataract formation that may require cataract surgery (implantation of an intraocular lens (IOL)) are not well understood in comparison to the mature and ever-improving surgical technology and procedures. The **hypothesis of the research** is that an ultrastructurally-based computational finite element model of the ocular lens subjected to blast loading can assist in better understanding how traumatic cataract is formed in the combat soldier, and in turn improve our understanding of traumatic cataract in civilians whose eyes are subjected to impact loading. The **scope of the research** is to develop a multi-scale, ultrastructurally-based, computational model of the ocular lens subjected to blast loading, in conjunction with imaging methods to identify lens capsule and internal substance structure and mechanical experiments for calibrating material model parameters.

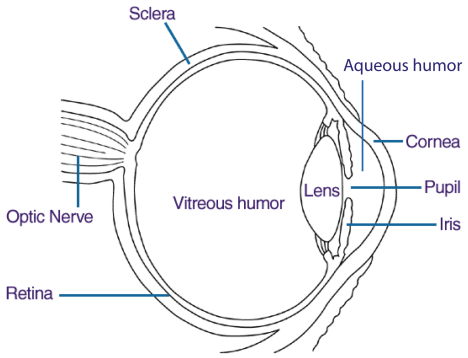

Figure 1. Eye cross-section. `www.nei.nih.gov`.

2 Body

The research tasks focussed on over the past year have been the imaging ones (Task 6), and some modeling (Task 1-3). For Task 6, undergraduate students Sai and Sri Radhakrishnan have taken the lead on the cryo-electron microscopy and tomography (cryo-EM and ET) (Sai) imaging of type IV collagen structure, and confocal laser scanning microscopy (CLSM) (Sri) for the internal lens fiber cells. In addition, on the modeling side a PhD student, Boning Zhang, has been recruited to work on Tasks 1-3. New results for a combined Eulerian-Lagrangian approach to large deformational loading of the lens, and solid-fluid interaction, are presented in collaboration with Assistant Professor Franck Vernerey and his PhD student, Louis Foucard.

A No Cost Extension (NCE) will be filed in May 2014, to extend the project through September 2016.

Next, research on the Tasks over the past year are summarized.

6a. Before and after whole porcine lens unconfined compression, image lens fiber cell geometry using CLSM (low strain rate subtask 4a: months 1-4; higher strain rate subtask 4b: months 6-8).

In collaboration with Dr. Christopher English of the Molecular, Cellular, and Developmental Biology (MCDB) department, we are still refining the procedure of CLSM for identifying the ultrastructure, at various cross-sections through the whole lens, of the internal lens fiber cells. We are focussing our efforts on as-received porcine lenses and one human lens.

We have conducted preliminary analysis on confocal images of human interior lens substance, attempting to identify the ultrastructure of the lens fiber cells. The first image of the slice, and the location of the slice in the context of a drawing of the lens fiber cell ultrastructure (drawing from pg99 of [Kessel and Kardon, 1979]) is shown in Fig.2. The donor was a 66-year-old male, with lens obtained in Balanced Salt Solution (BSS) within 48hrs post mortem. When the lens was prepared for confocal laser scanning microscropy (refer to Section 7.1 of Christopher Bays MS thesis, included as an attachment to Year 2 Final Report), the nucleus and cortex separated, and thus in the image only the cortex section is shown.

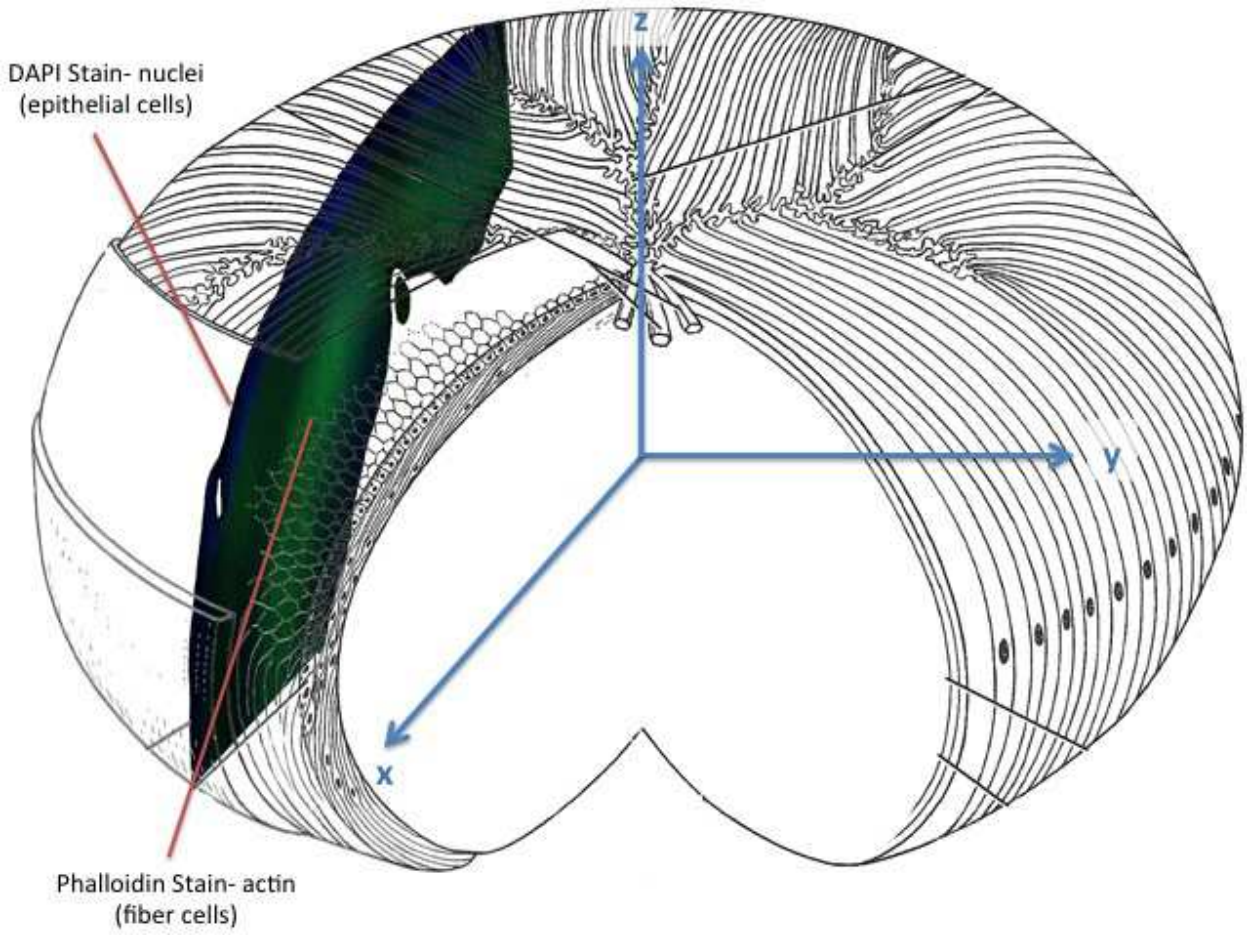

Figure 2. CLSM slice of human lens fiber cell structure aligned with approximate location within full lens (drawing from pg99 of [Kessel and Kardon, 1979]).

Figure 3 shows zoomed-in images of the lens fiber cell ultrastructure. When compared to images for the 2+yr old pig lens (see Chapter 7 of Christopher Bays MS thesis, as attachment to the Year 2 Final Report), the 66-year-old male fiber cell ultrastructure is less identifiable. One hypothesis for this less-identifiable fiber cell ultrastructure in the older human lens is the wearing or degradation of the lens fiber cells over longer time. This hypothesis remains to be tested as more data are obtained for younger human lenses, and from other older human lenses.

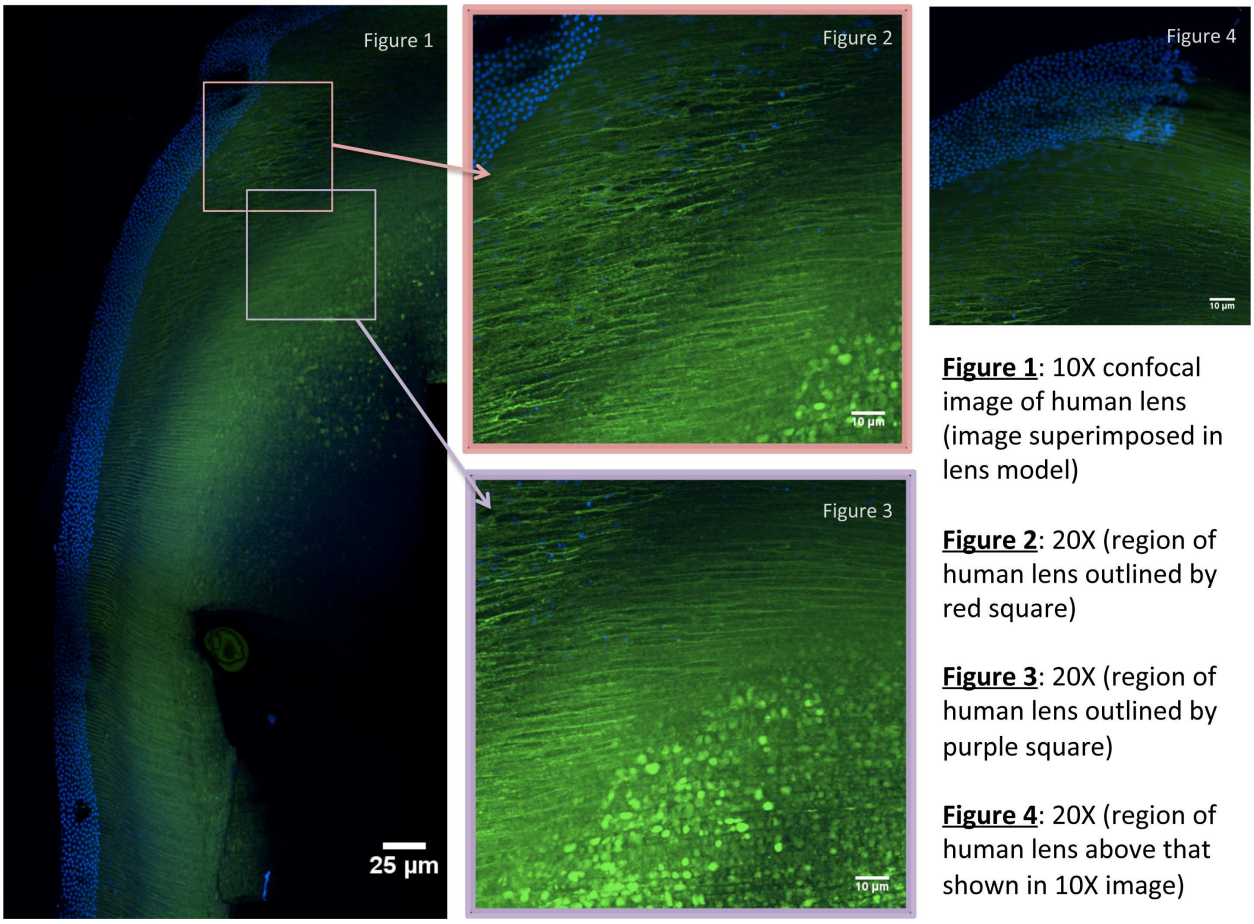

Figure 3. Zoomed-in images of CLSM slice of human lens fiber cell structure.

6b. On as-received porcine lens capsules, image type IV collagen ultrastructure in lens capsule using cyro-electron tomography (months 1-4).

Identification of type IV collagen meshwork ultrastructure in the lens capsule using cyro-electron microscopy/tomography is described in Chapter 6 of C. Bay's thesis, included as Appendix to last year's annual report. The current focus of the research is to determine if the images we are obtaining, such as in Fig.4, are indeed structure, or if they are an artifact of the freezing process. We are collaborating with Dr. Thomas Giddings and Dr. Andreas Hoenger of the Molecular, Cellular, and Development Biology (MCDB) Department at the University of Colorado, Boulder, about this topic. More experiments are being conducted to determine whether what we are seeing is structure or artifact, such as increasing the percentage of cryo-protectant solution.

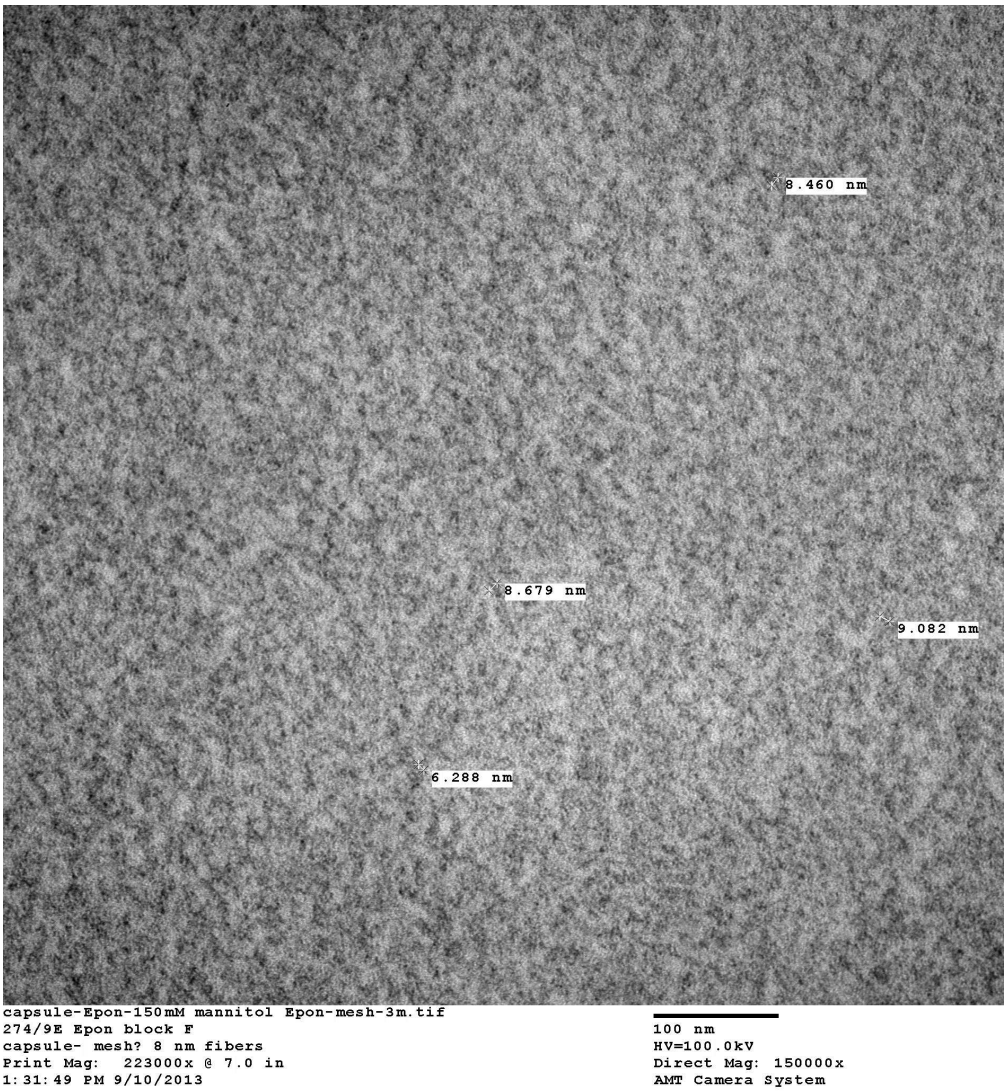

Figure 4. Cryo EM image of what is possibly type IV collagen structure.

2c: Formulation and finite element implementation of multiscale perforating finite strain biphasic mixture (solid and fluid) solid-shell continuum model of lens capsule in Tahoe, to model cutting/ perforation of lens capsule based on implementation in subtasks 1c and 2b (months 28-36). 3b: Using result of subtask 3a, formulate and implement multiscale hierarchical, anisotropic, lens fiber cell equivalent soft viscoelastic constitutive model of the internal lens substance (months 40- 48).

Large Deformation Lagrangian-Eulerian Computational Modeling of Lens Puncture and Also Solid-Fluid interaction between Lens and Surrounding Fluids:

For computational modeling, we are attempting a large deformation hybrid Lagrangian-Eulerian simulation of lens puncture tests in order to eventually model penetration by Intra-Ocular Foreign Bodies (IOFBs) (Secondary Blast), but also shock propagation and solid-fluid interaction between the lens and vitreous and aqueous humors (Primary Blast). This is in collaboration with Assistant Professor Franck Vernerey, and his PhD student Mr. Louis Foucard, both at CU Boulder. The images below illustrate a preliminary simulation of spherical tip indentation into a 2+yr old pig lens.

The method takes an Eulerian approach to describe the large deformations of an elastic membrane and its interactions with the surrounding fluid. The membrane is modeled as a two-dimensional elastic surface, across which discontinuities of continuum fields such as pressure and fluid velocity in the tangential direction can naturally be enforced using the extended finite element method (X-FEM). The tracking and evolution of the membrane is handled with the Grid Based Particle method that is well suited to evaluate the membrane higher order geometrical information.

The results of the simulation of indentation of a pig lens by a spherical indenter are compared with experimental data. The experimental data are obtained for a 2+ year-old porcine lens undergoing indentation by a 2mm (in diameter) indenter with a spherical tip (Fig.5). The indenter is lowered at a speed of 0.5mm/s until failure/puncture of the capsule. The simulation was carried out using a newly developed Eulerian approach able to describe the large deformations of an elastic membrane and its interactions with the surrounding and enclosed fluid (Fig.5). The capsule membrane is assumed to be incompressible and isotropic, for the time being. The Mooney-Rivlin strain-energy function was chosen to model membrane elastic response, in Eq.(1)

$$W = \frac{\mu}{2(1+\alpha)}\left[(1-\alpha)(\lambda_1^2+\lambda_2^2+\lambda_1^{-2}\lambda_2^{-2}-3)+\alpha(\lambda_1^2+\lambda_2^2+\lambda_1^2\lambda_2^2-3)\right] \qquad (1)$$

where μ is the infinitesimal shear modulus of the elastic material, α is a parameter that controls the deviation from a linear Hookean response, and λ_1 and λ_2 are the principal stretches in direction 1 (radial) and 2 (axial) . In the simulation, the capsule is considered filled with a Newtonian viscous fluid of viscosity μ_f.

In this simulation, the indenter (black cylinder) in Fig.5 progressively approaches and deforms the elastic capsule filled with a viscous fluid and resting on a plane surface. The initial geometry of the eye lens was extracted from the experimental video of indentation at time $t = 0$, and the system was meshed accordingly using a 23x30 element grid. The (friction-less) interaction between the capsule and the indenter is handled via the penalty method. Figure 6 shows the dilatation (jacobian J) of the elastic membrane. Figure 6 shows the deformation gradient components in the axial (F_{22}) and radial (F_{11}) directions. One can observe the very large deformation (approaching 800%) and

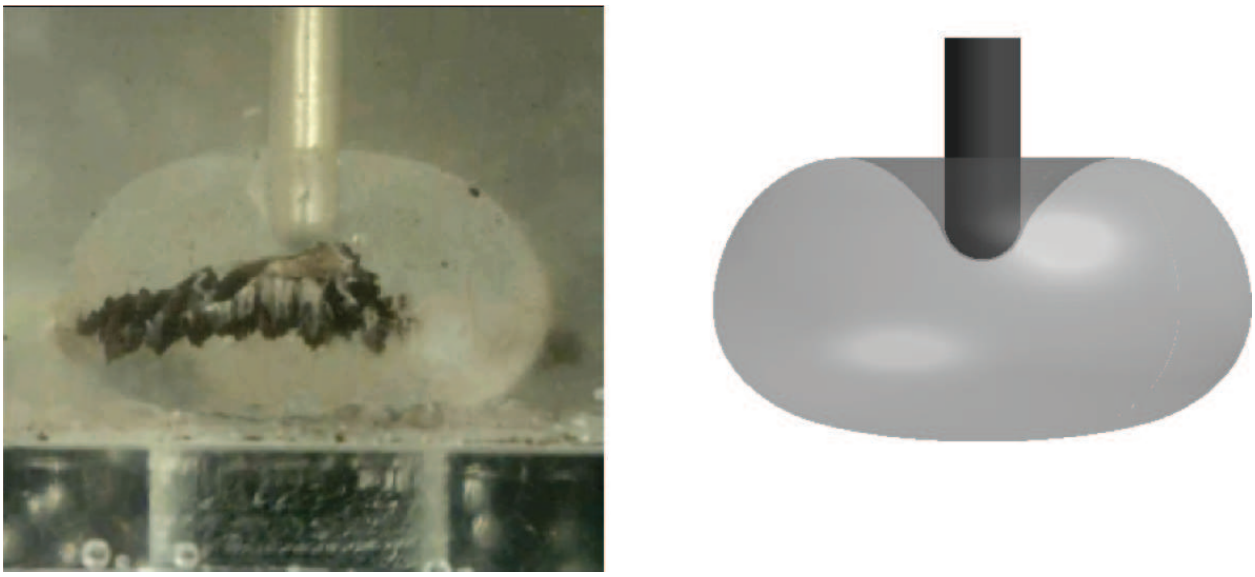

Figure 5. Indentation and large deformations of the porcine (2+years old) lens: (left) experiment, (right) simulation. The indenter has a 2mm diameter and a spherical tip and is lowered at 0.5mm/s until puncture of the capsule.

dilatation of the membrane at the tip of the indenter, which would prove very difficult to simulate using a Lagrangian approach. This stress concentration at the tip of the indenter is also consistent with the fact that the rupture of the capsule membrane was observed to take place at the same location experimentally. Finally, Fig.6 shows the normalized force versus displacement curve for both the simulation and the experimental results. Up to the point of failure of the capsule (peak), the simulation results show good consistency with the experimental data.

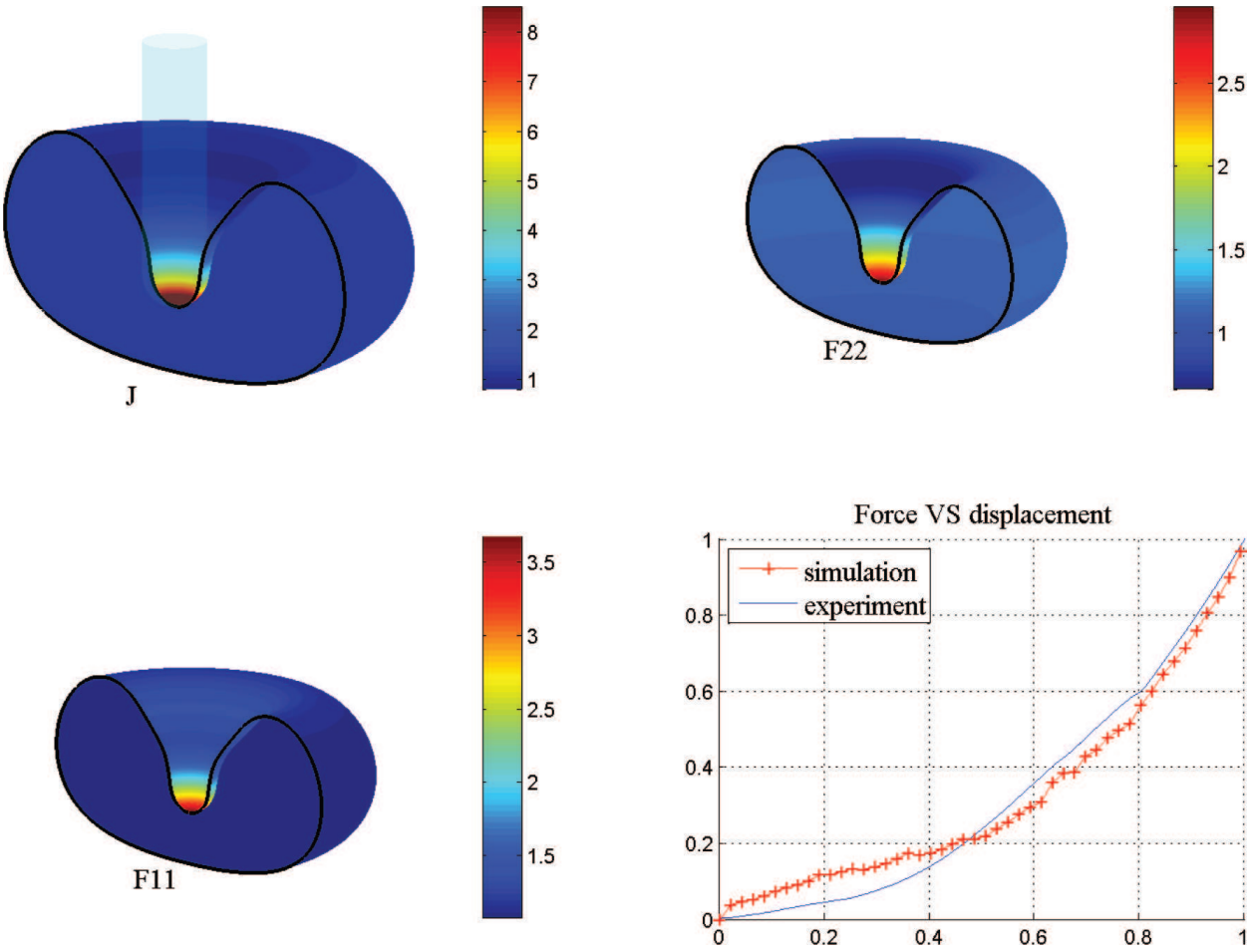

Figure 6. Indentation and large deformations of lens. (top left) dilatation of the surface (Jacobian), while (top right) and (bottom left) show deformation gradient in axial (F_{22}) and radial (F_{11}) directions. (bottom right) normalized force versus displacement curve for both the simulation results and the experimental data.

3 Key Research Accomplishments

- CLSM images of the internal lens fiber cell structure are showing promise, and we are confident we can obtain good structural identification of lens fiber cell structure pre and post-test. This will be used to identify the lens fiber cell structure for the multiscale modeling of the internal lens substance.

- Electron Microscopy (EM) and Tomography (ET) images are not yet believed to be structure, but could possibly be an artifact of the freezing process. This is beginning to raise questions as to the validity of type IV collagen structural images provided in the literature to date. The MCDB department and Bio3D lab `bio3d.colorado.edu` are world-renown, NIH-funded facilities for identifying structure of biological tissues. Thus, they have excellent facilities, and are careful in their tissue preparation and interpretation of results.

- Preliminary large deformation Lagrangian-Eulerian axisymmetric computational modeling of lens indentation, and also solid-fluid interaction between the lens and surrounding fluids, shows promise for providing a robust large deformation computational mechanics framework for simulating Primary and Secondary blast on the lens, as well as the whole eye globe, potentially.

4 Reportable Outcomes

1. August 2013, Experiments and Finite Element Analysis of Stress Relaxation upon Compression of Whole Porcine Lenses, *EMI 2013*, Northwestern University.

2. On June 24-25, Dr. Matthew Reilly from the University of Texas, San Antonio (UTSA), visited PI Regueiro to discuss each other's research and how we can work together.

3. The collaboration beginning with Dr. Vernerey of CU Boulder on large deformation Lagrangian-Eulerian computational modeling of lens indentation, and also solid-fluid interaction between the lens and surrounding fluids, shows promise for providing a robust large deformation computational mechanics framework for simulating Primary and Secondary blast on the lens, as well as the whole eye globe, potentially.

5 Conclusion

The research progress over the last year has focussed on imaging of the lens fiber cell structure using confocal laser scanning microscopy (CLSM), and type IV collagen structure using cryo-electron microscopy and tomography (cryo-EM and ET). The CLSM promises to generate good structural identification of lens fiber cells, while the cryo-EM and ET have not yet positively identified type IV collagen structure of the lens capsule.

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